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Ontogeny of Osmoregulation and Salinity Tolerance in Two Decapod Crustaceans: *Homarus americanus* and *Penaeus japonicus*

G. CHARMANTIER[1], M. CHARMANTIER-DAURES[1], N. BOUARICHA[1],
P. THUET[1], D. E. AIKEN[2], AND J.-P. TRILLES[1]

[1]*Laboratoire de Physiologie des Invertébrés, Université des Sciences et Techniques du Languedoc,
Pl. E. Bataillon, 34060 Montpellier Cédex, France, and* [2]*Department of Fisheries and Oceans,
Invertebrate Biology and Aquaculture, Biological Station, St. Andrews,
New Brunswick E0G 2X0, Canada*

Abstract. Osmoregulation and salinity tolerance were studied in larvae and post-larvae of two species of crustaceans, *Homarus americanus* and *Penaeus japonicus*, that have different types of embryonic development.

In both species, salinity tolerance decreased through the larval stages, was at a minimum at metamorphosis, and increased in post-larval stages. In *H. americanus*, the lethal salinity for 50% of the animals (24 h LS50) at 20°C was about 17‰ at metamorphosis, and about 10.5–12‰ in stages IV and V. In *P. japonicus*, the 24 h LS50 at 25°C was about 25‰ at metamorphosis, and about 7–10‰ from the sixth post-larval stage onwards.

In both species, larvae were hyper-osmoconformers and the osmoregulatory pattern changed after metamorphosis to the juvenile/adult type. In *H. americanus*, stages IV and V slightly hyper-osmoregulated in low salinities. In *P. japonicus*, post-larvae hyper-hypo-regulated, and their regulatory capacity increased up to the fifth post-larval stage.

In young stages of *H. americanus* and *P. japonicus*, osmoregulation and salinity tolerance appear correlated, and are modified at metamorphosis. These results are discussed with regard to their ecological and physiological implications and to previous studies on other species.

Introduction

Most studies on crustacean osmoregulation deal with adult forms (review in Mantel and Farmer, 1983), and only a few data are available on larval and post-larval osmoregulation. These are summarized in Table I. Depending on species, the osmoregulatory abilities can vary among the successive larval stages or remain unchanged. The adult type of regulation is also established at variable stages in different species. Numerous studies have been concerned with larval and post-larval salinity tolerance, but few have attempted to correlate the salinity tolerance of different developmental stages with their corresponding osmoregulatory capabilities in a given species.

The objective of this study conducted with the American lobster *Homarus americanus* H. Milne Edwards, 1837, and the shrimp *Penaeus japonicus* Bate, 1888, was to determine the salinity tolerance of larval and post-larval stages of these species, to define the ontogeny of their osmoregulation, and to attempt to correlate osmoregulatory abilities and salinity tolerance. *H. americanus* and *P. japonicus* are both economically important, and any knowledge of their larval and post-larval environmental tolerance and physiology can be valuable for their management and potential culture. Moreover, their patterns of post-embryonic development are different, offering the opportunity for comparisons of larval physiology.

In the genus *Homarus*, larval development comprises one prelarva and three zoea or mysis larvae—stages I to III—before a metamorphosis leading to post-larvae—stage IV or megalopa—and then to juvenile stages. In *H. americanus*, osmoregulation has been studied in adults (Dall, 1970) and juveniles (Charmantier *et al.*, 1981, 1984a); information on ionic regulation (Charmantier *et al.*, 1984b) and preliminary data on osmoregulation (Charmantier *et al.*, 1984c) are also available for the early post-embryonic stages of this species. Osmotic and ionic

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Table I

Summary of studies on larval/post-larval crustacean osmoregulation

Species	Larval/ post-larval development	Type(s) of osmoregulation during post-embryonic development	Type of osmoregulation in adults
Rhithropanopeus harrisii (1)	4 zoeae	Hyper-osmoconform from 10 to 40 ‰ Slight hyper-regulation in lowest salinities. Except osmoconform in diecdysial stage V	Hyper-hypo-regulation (2)
Cardisoma guanhumi (3)	5 zoeae	Hyper-osmoconform <20 ‰ Tendency to hyper-regulate in 30–40 ‰ in 3rd, 4th, 5th zoeae	Hyper-hypo-regulation (4) (5)
Callinectes sapidus (6)	7 zoeae several megalopae	Hyper-isoregulation in 1st, 2nd zoeae and late megalopa. Osmoconform in 7th zoea. Hyper-osmoconform in other stages	Hyper-hypo-regulation (7)
Hepatus epheliticus (6)	3 zoeae	Hyper-isoregulation in 1st zoea. Slightly hypo-regulate in 40 ‰ in following stages. Larvae approaching megalopa and settling crab stages gradually osmoconform	Supposedly osmoconform (6)
Libinia emarginata (6)	2 zoeae 1 megalopa	1st zoea and late megaloga osmoconform. Slight hypo-regulation in 40 ‰ in 2nd zoea and early megalopa	Osmoconform (6) (8)
Sesarma reticulatum (9)	3 zoeae 1 megalopa	Hyper-regulate from 10–35 ‰. Slight hyper-regulation in 40 ‰	Hyper-hypo-regulation (9)
Clibanarius vittatus (10)	5 zoeae 1 megalopa	Hyper-osmoconform from 200 to 1200 mosm · kg^{-1}	Hyper-regulation Isosmotic regulation (11)
Macrobrachium petersi (12)	9 larval stages	Hyper-hypo-regulation Stronger ability in 1st stage and in post-larvae	Hyper-hypo-regulation (12)
Uca subcylindrica (13)	2 zoeae 1 megalopa	Slight hyper-regulation in zoeae I–II Slight hyper-hypo-regulation in megalopa	Hyper-hypo-regulation (13)
Callianassa jamaica louisianensis (14)	2 zoeae	Slight hyper-regulation in media <800–900 mosm · kg^{-1}	Hyper-hypo-regulation (15)

(1) Kalber and Costlow (1966)
(2) Smith (1967)
(3) Kalber and Costlow (1968)
(4) Pearse (1932)
(5) Quinn and Lane (1966)
(6) Kalber (1970)
(7) Ballard and Abbott (1969)
(8) Gilles (1970)
(9) Foskett (1977)
(10) Young (1979a)
(11) Young (1979b)
(12) Read (1984)
(13) Rabalais and Cameron (1985)
(14) Felder *et al.* (1986)
(15) Felder (1978)

regulation of *H. gammarus* has been studied in juveniles (Charmantier *et al.*, 1984d).

In contrast to the simple post-embryonic development of homarid lobsters, the penaeid shrimps have numerous larval instars beginning with nauplii stages, which is an exception among decapod crustaceans. The post-embryonic development of *Penaeus japonicus* goes through six nauplius stages, three zoeae, and three mysis. The third mysis stage ends with a metamorphosis: the succeeding post-larval stages can be named either by their stage (*e.g.*, PL5, fifth post-larva) or by the time elapsed since metamorphosis (*e.g.*, P5, 5 days after metamorphosis). Although osmoregulation has been studied extensively in penaeid shrimps (review in Charmantier, 1987b), only ionic regulation is known in *P. japonicus* (Exbrayat and Bourguet, 1982). Preliminary data have also been gathered on osmoregulation of their larvae (Charmantier, 1986).

Materials and Methods

This study was conducted at two different locations: St. Andrews (New Brunswick, Canada) for *Homarus americanus* and Montpellier (Hérault, France) for *Penaeus japonicus*. Standardized methods were used for both species and two of us were members of both the

Canadian and French groups who performed the experiments.

Animals

Homarus americanus larvae were obtained during the summer from lobsters captured in Passamaquoddy Bay and held in the lobster culture facility at the Biological Station at St. Andrews. After hatching, larvae were transferred to 40-l planktonkreisels (Hughes *et al.*, 1974) supplied with flow-through seawater at a salinity of 30–31‰, a temperature of 20°C under natural photoperiod. Larvae were fed three times a day with frozen adult *Artemia*. As each larval stage lasts several days, molting stages were obtained according to the time elapsed from the preceeding molt, and three groups of animals, postmolt stage A, stage C and premolt stage D, were selected.

Larvae of *P. japonicus* were obtained in early spring from the Ifremer Station (Deva-Sud) at Palavas (Hérault). They were reared in semi-recirculated systems using a clear water technique at a salinity of 35–36‰, a temperature of 25–27°C and under an artificial photoperiod (12L/12D) (Aquacop, 1983; Laubier, 1986). Larvae were fed with algae or *Artemia* nauplii according to their stage. Each larval stage lasted 24 h or less, so it was not possible to select animals according to molting stages.

Preparation of media

Dilute media were prepared by addition of tap water to seawater and high salinity media were prepared by adding "Instant Ocean Synthetic Sea Salts" (Aquarium Systems, Inc.) to seawater. All experiments were conducted at 20°C (*Homarus*) or 25°C (*Penaeus*). Salinities were expressed according to the osmotic pressure in mosm $\cdot$ kg^{-1} and to the salt content of the medium in ‰. Osmotic pressure was measured on an Advanced Instruments 31 LA or Roebling osmometer, and salinity on a YSI 33 salinometer. A value of 3.4 ‰ is equivalent to 100 mosm $\cdot$ kg^{-1}.

Survival bioassays

To determine salinity tolerance, acute static bioassays were conducted with animals held in test media ranging from fresh water (~10 mosm $\cdot$ kg^{-1}) to seawater (~900–1100 mosm $\cdot$ kg^{-1}) and differing by increments of 100 mosm $\cdot$ kg^{-1} (= 3.4‰). Penaeid shrimp larvae were held communally in plastic containers holding 0.5 l of medium; due to agonistic behavior, lobster larvae were held in individual compartments partly immersed in 3.5-l containers. All media were aerated. Animals were not fed during the bioassays. The duration of lobster experiments was 48–96 h. The shrimp experiments lasted 24–96 h due to the shorter duration of the stages.

Each bioassay was run on a group of 10 animals and replicated. Animals were counted and dead animals removed at 0.5, 1, 2, 3, 6, 12, 24, and 48 or 96 h according to the prescriptions of Sprague (1969) in toxicity studies. The criteria for death were total lack of movement, immobility of the scaphognathite (lobster) and of the heart (both species), and lack of response after repeated touches with a probe. Median times to 50% mortality (LT50) and their 95% confidence limits were determined from a computer program (Lieberman, 1983) based on the probit technique of Lichtfield and Wilcoxon (1949) and Finney (1962). Median lethal salinities (LS50) and 95% confidence intervals were calculated by standard techniques of probit analysis (Finney, 1962; Davies, 1971) computerized on the Letcur program (Zitko, 1982). LS50 were calculated at 24 and 48 h, and at 96 h in some longer lasting post-larval stages.

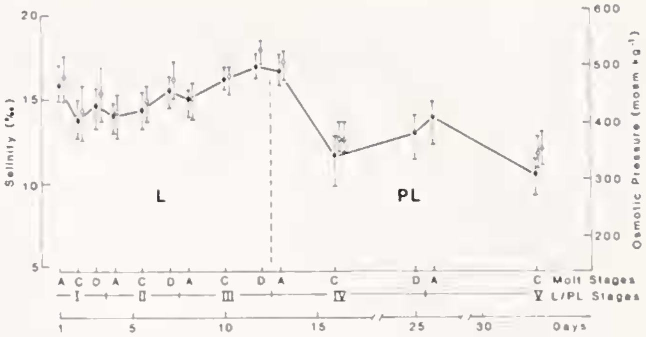

Figure 1. Salinity tolerance in larval (I, II, III) and post-larval (IV, V) *Homarus americanus* at 20°C. Variations in LS50 in ‰ and mosm $\cdot$ kg^{-1} according to larval (L)/post-larval (PL) and molt stages and to days of development. Each point represents the mean value of at least two determinations from 10 animals, with 95% confidence interval. Closed circles: 24 h LS50; open circles: 48 h LS50; open triangles: 96 h LS50.

Osmoregulation

Animals were reared at different selected salinities in recirculated planktonkreisels (lobster) or 0.5-l plastic containers (shrimp). Individuals were dried on filter paper and hemolymph was sampled by inserting a micropipette into the heart. This operation was conducted under mineral oil in the smallest stages in order to avoid rapid evaporation and desiccation. In shrimp, reproducible data were obtained only from stage zoea 2, *i.e.*, more than 1.3 mm long. Osmotic pressure of hemolymph was measured on a Kalber-Clifton micro-osmometer requiring 30-50 nl, with reference to the osmotic pressure of the medium.

Results

Homarus americanus: *salinity tolerance*

The ability of *H. americanus* to tolerate low salinities varied with post-embryonic development (Fig. 1). After a slight decrease early in stage I, the 24 h LS50 increased through stages I, II, and III from about 410 mosm · kg^{-1} (14‰) to a maximum (corresponding to a minimum tolerance) of 500 mosm · kg^{-1} (17‰) in stages III D and IV A–B preceding and following metamorphosis. In postlarval stages IV and V (in molting stage C), salinity tolerance reached its maximum, with 24 h LS50 values down to 340 mosm · kg^{-1} (11.6‰) and 310 mosm · kg^{-1} (10.5‰); however, LS50 increased during the molt from stage IV to stage V. The 48-h LS50, although higher by 10–40 mosm · kg^{-1} (0.3–1.4‰), followed the same pattern of variation. Molting between larval stages was possible in respectively 0%, about 50%, and more than 80% of the larvae in media of ≤400, 500–600 and ≥700 mosm · kg^{-1} (13.6, 17–20.4, 23.8‰).

H. americanus: *osmoregulatory ability*

Adaptation time. After a rapid transfer from seawater at 850 mosm · kg^{-1} (29‰) to a dilute medium of 500 mosm · kg^{-1} (17‰), the hemolymph osmotic pressure stabilized within 1 h in stages I and II, 2 h in stage III and 3–6 h in stage IV (Fig. 2). Osmotic adaptation to concentrated media of 1100 and 1300 mosm · kg^{-1} (37 and 44‰) was completed in 1–3 h. In all subsequent experiments we kept the animals 6–24 h in each medium before sampling.

Osmoregulation. The types of osmoregulation were similar in larval stages I, II, and III. In molting stage C, these larvae hyper-osmoconformed over the whole range of salinity, the osmotic pressures of hemolymph and medium differing by about 10–20 mosm · kg^{-1}. Their regulation was slightly more hyper-osmotic in premolt and nearly isosmotic in post-molt (Fig. 3).

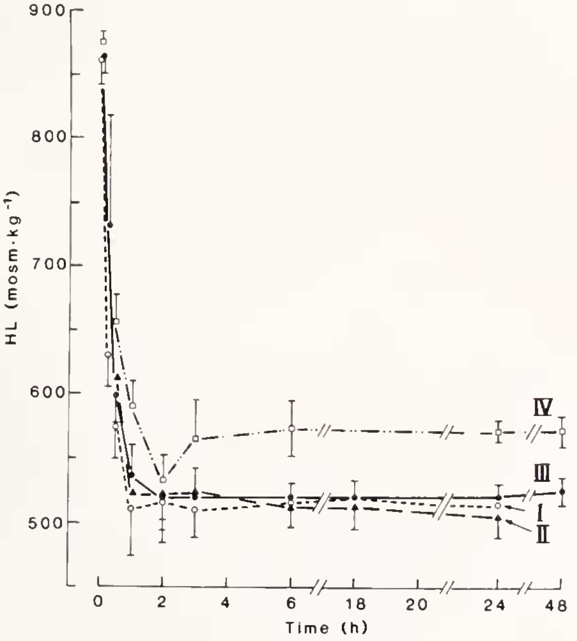

Figure 2. Change in hemolymph osmotic pressure (HL) in stages I–IV of *Homarus americanus* after rapid transfer from seawater (850 mosm · kg^{-1}, 29‰) to a dilute medium (500 mosm · kg^{-1}, 17‰) at 20°C. Each point represents the mean value of determinations from 12–15 animals, with 95% confidence interval.

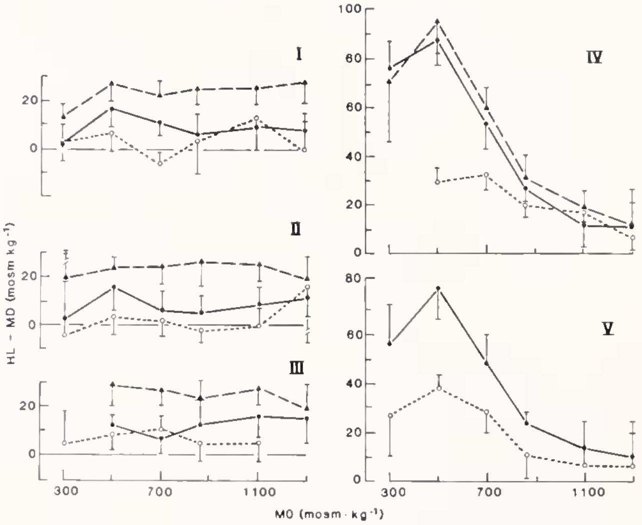

Figure 3. Variations in the difference between the osmotic pressures of hemolymph and medium (HL-MD) according to the osmotic pressure of the medium (MD) in stages I–V of *Homarus americanus* at 20°C. Each point represents the mean value of determinations from 12–20 animals (except in extreme salinities: 5–16 animals) with 95% confidence interval. ○ ---- ○: post-molt; ●——●: stage C; ▲ - - - ▲: premolt.

The pattern of osmoregulation changed after metamorphosis. Stage IV and V post-larvae in molting stage C hyper-osmoconformed in high salinities and seawater and their regulation was slightly hyper-osmotic in dilute media (hemolymph-medium difference of about 80 mosm · kg^{-1} in a 500 mosm · kg^{-1} or 17‰ medium). No significant difference was found in the regulation of premolt post-larvae, but the ability to hyper-regulate in dilute media significantly decreased in post-molt stages IV and V (hemolymph-medium difference of about 30–35 mosm · kg^{-1} in a 500 mosm · kg^{-1} or 17‰ medium) (Fig. 3).

Penaeus japonicus: *salinity tolerance*

Tolerance of low salinities varied with the developmental stages of *P. japonicus* (Fig. 4). The 24 h LS50 increased during larval development from about 460–600 mosm · kg^{-1} (16–20‰) in nauplii and zoeae 1 up to 730 mosm · kg^{-1} (25‰) just prior to and after metamorphosis in mysis 3 and first post-larval stages (PL1). Salinity tolerance increased progressively thereafter up to stage PL6, P12, *i.e.*, 12 days after metamorphosis (24 h LS50 about 300 mosm · kg^{-1} or 10‰) and more slowly up to stage

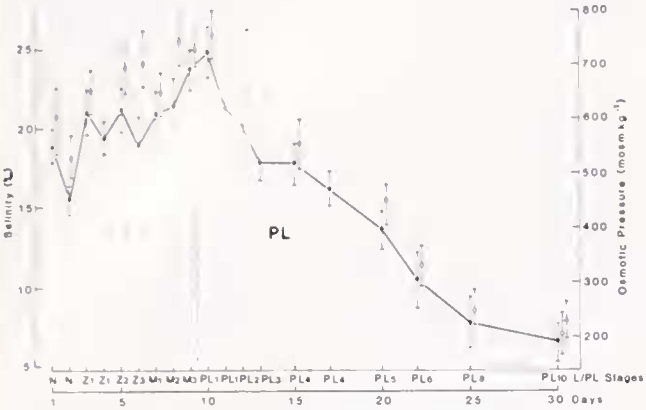

Figure 4. Salinity tolerance in larval (L) and post-larval (PL) *Penaeus japonicus* at 25°C. Variations in LS50 in ‰ and mosm · kg^{-1} according to larval/post-larval stages and days of development. Each point represents the mean value of at least two determinations from 10 animals, with 95% confidence interval. N, nauplius; Z, zoea; M, mysis; Pl, post-larval stage. Closed circles: 24 h LS50; open circles: 48 h LS50; open triangles: 96 h LS50.

PL10, P20 (24 h LS50 about 200 mosm · kg^{-1} or 7‰). The 48 h LS50, higher than the 24 h LS50 by 20–150 mosm · kg^{-1} (0.7–5.1‰), followed the same pattern of variation. Molting between larval stages was possible in 0%, about 50%, and 60–100% of the larvae in media of ≤400, 600–700, and ≥800 mosm · kg^{-1} (13.6, 20.4–23.8, 27.2‰), respectively.

P. japonicus: *osmoregulatory ability*

Adaptation time. After a rapid transfer from seawater (1050 mosm · kg^{-1} or 35.5‰) to a dilute medium (500 mosm · kg^{-1} or 17‰), the hemolymph osmotic pressure stabilized in 1 h in stage zoea 3, 3 h in fourth post-larval stage (PL4), and 6 h in stage PL10 (Fig. 5). In all subsequent experiments, we kept the animals 6–24 h in each medium before sampling.

Osmoregulation. Larval stages (zoea 2 and 3, mysis 1–3) hyper-osmoconformed over the whole range of tested salinities: the osmotic pressures of hemolymph and medium differed by about 10–40 mosm · kg^{-1}. In stage mysis 3, which lasted 2 days, regulation was slightly more hyper-osmotic towards the end of the stage, *i.e.*, in premolt animals (Fig. 6). At the end of the larval period, most of the animals died in the dilute media and the few survivors tended to osmoconform.

The pattern of osmoregulation changed after metamorphosis. Starting from the end of first post-larval stage (PL1) which lasted 2 days, the regulation shifted to slightly hyper-osmotic in dilute media and hypo-osmotic in seawater. Hyper- and hypo-osmoticity increased progressively thereafter up to PL5–PL6, P10–P12. From these stages onwards, the difference between the osmotic pressures of hemolymph and media reached about 300, 200, and −150 mosm · kg^{-1} in 300, 500, and 1000 mosm · kg^{-1} media (corresponding salinities: 10, 17, 34‰), respectively. The isosmoticity medium changed progressively from 900 mosm · kg^{-1} (30.5‰) in PL1 to 800 mosm · kg^{-1} (27‰) in PL5–PL6 (Fig. 6).

Discussion

Salinity tolerance

In *Homarus americanus*, salinity tolerance at 20°C expressed by the 24 h LS50 varies from 14–17‰ in larvae down to 10.5–12‰ in post-larvae and is minimum at metamorphosis—about 17‰. These results are in agreement with previous data: in *H. gammarus* and *H. americanus*, respectively, Gompel and Legendre (1927) and Templeman (1936) found that the larval period could progress to metamorphosis and stage IV only at 15–17.5°C in salinities above 17‰. Sastry and Vargo (1977) observed that larvae developed to stage V in salinities above 20‰ at 15°C and 15‰ at 20°C. Thus, from these studies, the minimum salinity compatible with larval development and metamorphosis can be estimated to be

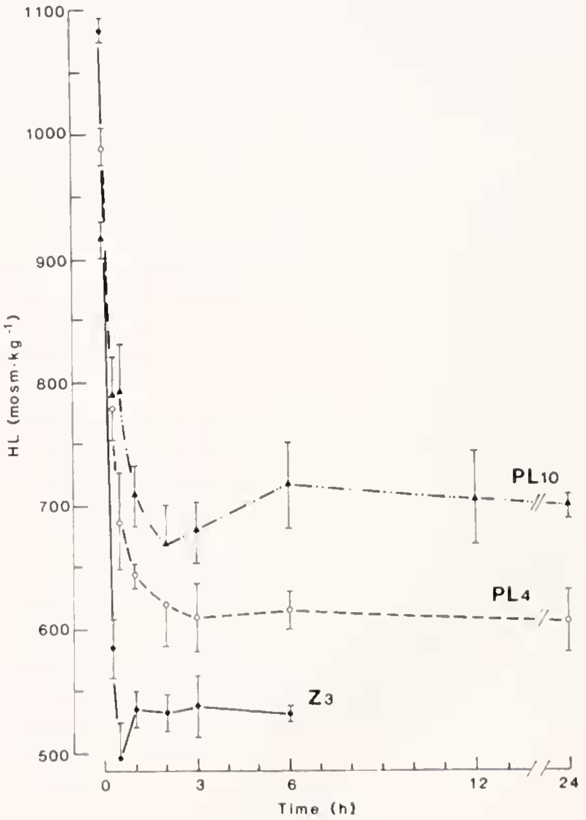

Figure 5. Change in hemolymph osmotic pressure (HL) in stages zoea 3 and post-larvae 4 and 10 of *Penaeus japonicus* after rapid transfer from seawater (1050 mosm · kg^{-1}, 35.5‰) to a dilute medium (500 mosm · kg^{-1}, 17‰) at 25°C. Each point represents the mean value of determinations from 10–15 animals, with 95% confidence interval.

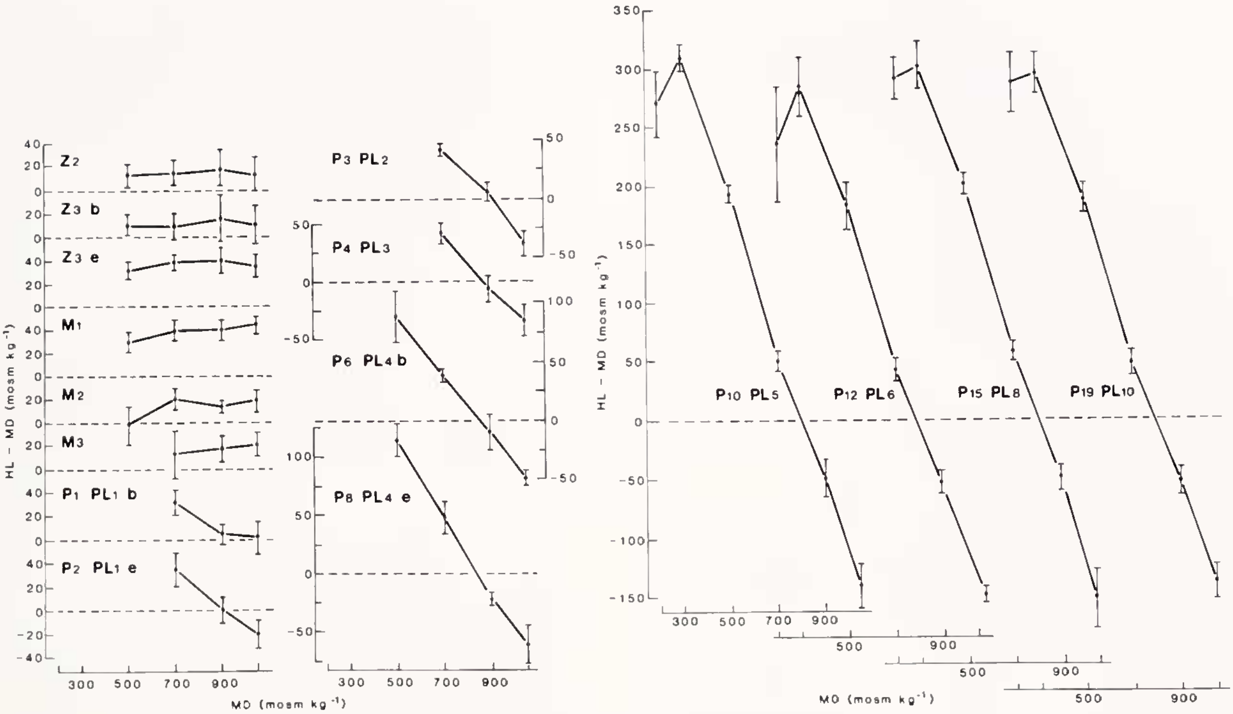

Figure 6. Variations in the difference between the osmotic pressures of hemolymph and medium (HL-MD) according to the osmotic pressure of the medium (MD) in larval and post-larval stages of *Penaeus japonicus* at 25°C. Each point represents the mean value of determinations from 10–15 animals (except in some low salinities, 5), with 95% confidence interval. Z, zoea; M, mysis; P, number of days after metamorphosis; PL, post-larval stage; b, beginning; e, end.

about 17‰ at 20°C. After metamorphosis, the values of LS50 found in post-larvae are similar to those observed in one-year-old juveniles (10‰: Charmantier, unpub. data) and compatible with the lethal limits known in adults (8‰: McLeese, 1956). The salinity tolerance of post-larvae decreases at the time of molt, which is a frequent observation among crustaceans.

In *P. japonicus,* the 24-h LS50 at 25°C varies from 16–20‰ to 25‰ in larval stages with a maximum value at metamorphosis. It decreases progressively to 10‰ from post-larval stage PL1 to PL6, and to 7‰ in stage PL10. In *P. japonicus* (Hudinaga, 1942), *P. duorarum* (Ewald, 1965), *P. marginatus* (Gopalakrishnan, 1976), and *Metapenaeus bennettae* (Preston, 1985), zoea larvae are less resistant to low salinity than mysis, which is not the case in our study. There are two possible explanations of this. The different feeding conditions could interfere with the effect of salinity. Preston (1985) observed that "starvation was a more potent factor than the effects of temperature and salinity in determining survival through the protozoeal larval stages." On the other hand, most of these studies were conducted for long periods to complete the larval development, which is not the case in our stage-by-stage study. After metamorphosis, the values of LS50 from PL6-PL8 were similar to those found in juveniles of the same species (8‰: Dalla Via, 1986; 6‰: Thuet *et al.*, unpub. data).

In *H. americanus* and *P. japonicus,* the 48 or 96 h values of LS50 are higher than those at 24 h. The difference is highest in the stages that last less than 48 h (*Penaeus* larval stages, young *Homarus* in molting stage D). High mortality occurs in the lowest salinities when these animals attempt to molt.

Adaptation time

In *H. americanus* and *P. japonicus* the time required for osmotic equilibration to a dilute medium is about 1–2 h in larvae, and 3–6 h in early post-larvae. It is between 12 and 24 h in juvenile lobster (Charmantier *et al.*, 1984a) and adult shrimp (Charmantier, unpub. data), and about 75 h in adult lobster (Dall, 1970). Therefore, adaptation time is size dependent. In larvae of other species, adaptation time is similar to that of young stages of *Homarus* and *Penaeus* (Kalber and Costlow, 1966, 1968; Kalber, 1970; Foskett, 1977; Felder *et al.*, 1986). Foskett (1977) stressed the physiological and ecological importance of rapid adaptation of hemolymph osmotic pressure to changes in the salinity of the medium. This may be particularly true in the case of larval *Homarus* and *Penaeus* which are planktonic and thus exposed to sudden changes in salinity following heavy rainfall. These rapid changes also require the existence of intracellular osmotic adaptation, especially in the hyper-osmoconforming larval stages of the two species.

Osmoregulation

In *H. americanus*, larval stages I–III hyper-osmoconform and post-larval stages IV and V slightly hyper-regulate in dilute media. The adult type of osmoregulation (Dall, 1970), which is identical to juvenile regulation (Charmantier *et al.*, 1981, 1984a), is acquired at stage IV, following metamorphosis. We found the same pattern of changes in the osmotic regulation of larvae and post-larvae of *H. gammarus* (Thuet *et al.*, 1988), confirming the physiological likeness of the two species.

The molt cycle affects osmotic regulation in *H. americanus* in two ways. In premolt larvae in stages I–III, the osmotic pressure of the hemolymph increases in all media. Similar variations have been shown in the larvae of *Rhithropanopeus harrisii* (Kalber and Costlow, 1966) and *Cardisoma guanhumi* (Kalber and Costlow, 1968). These authors suggested that a hyperosmotic internal medium would favor uptake of water at molt. In *H. americanus*, the tendency toward increased hyper-regulation before ecdysis is lost after metamorphosis, but postmolt post-larvae demonstrate a lower ability to hyper-regulate than do stage C animals. The decrease in hemolymph osmotic pressure or ion concentration is well known in postmolt adult crustaceans, where it is related to the water intake at molt, as exemplified by different lobsters (Travis, 1955; Glynn, 1968). Thus the effect of molting on osmotic regulation is different before and after metamorphosis. This could be related to variations in the permeability of the cuticle in larvae and post-larvae and to changes in the mechanisms involved, as discussed later.

In *P. japonicus*, zoeal and mysis larval stages hyper-osmoconform and the type of osmoregulation changes to hyper-hypo-regulation after metamorphosis. In post-larvae, the ability to regulate increases progressively up to stages PL5-PL6. In *P. japonicus*, the adult type of regulation is hyper-hypo-osmotic as in most species of penaeid shrimps (see review in Charmantier, 1987b); thus this type of regulation appears soon after metamorphosis but its efficiency is only gradually established over 10–12 days at 25°C.

A few studies have addressed the evolution of osmoregulatory abilities during the post-embryonic development of decapod crustaceans (Table I). In several species larvae are hyper-osmoconformers, for example *Rhithropanopeus harrisii* (Kalber and Costlow, 1966), *Callinectes sapidus* (Kalber, 1970), *Sesarma reticulatum* (Foskett, 1977), *Clibanarius vittatus* (Young, 1979a). *H. americanus* and *P. japonicus* larvae exhibit a similar type of osmoregulation. In other species such as *Cardisoma guanhumi* (Kalber and Costlow, 1968), *Hepatus epheliticus*, *Libinia emarginata* (Kalber, 1970), *Uca subcylindrica* (Rabalais and Cameron, 1985), *Callianassa jamaicense louisianensis* (Felder *et al.*, 1986), *Macrobrachium petersi* (Read, 1984), some capability for hypo- or hyper-regulation exists in certain larval stages. Thus, most decapod larvae that have been studied are osmoconformers or weak regulators. However, in species confronted with very low salinities in their natural environment, like *M. petersi*, larvae can efficiently regulate the osmotic concentration of their hemolymph.

In several of these species, the osmotic response varies little throughout the larval and post-larval development. Thus Foskett (1977) noted "no clear trend toward development of adult osmoregulatory patterns toward the end of larval life." However, recent findings and our own results contradict this generalization. Larvae of *M. petersi*, except in stage I, are incapable of osmoregulating in fresh water. This capacity re-appears in post-larvae, whose regulation is similar to that of juveniles and adults (Read, 1984). In *U. subcylindrica*, the hyper-hypo-regulation pattern of adults appears in megalopae and in the crab I stage (Rabalais and Cameron, 1985). The adult type of regulation is also present from post-larvae onwards in *H. americanus* and *P. japonicus*. Thus it can be stated that metamorphosis marks a profound change in the osmoregulatory abilities of these species. More generally, metamorphosis can be considered a combination of morphological, ecological, behavioral, and physiological changes (Charmantier *et al.*, 1984b; Charmantier, 1987a). In *P. japonicus*, the morphological metamorphosis is spread over several post-larval stages, which could be related to the progressive increase in osmoregulatory ability from PL1 to PL5. In *H. americanus*, on the contrary, both osmoregulatory and morphological changes are rapidly established in stage IV.

Na^+-K^+ ATPase is known to be implicated in ion transport. In *H. gammarus* the activation of this enzyme in dilute media is significantly higher in stage IV than in the preceding larval stages (Thuet *et al.*, 1988) which seems directly related to the hyper-regulation appearing from this stage onwards.

Evidence for neuroendocrine control of larval osmoregulation was first given by Kalber and Costlow (1966) in zoeae of *R. harrisii*. By eyestalk ablation and implantation the existence of neuroendocrine control of sodium regulation in *H. americanus* (Charmantier *et al.*, 1984b) and of osmoregulation in *H. americanus* and *H. gammarus* (Charmantier, unpub. data) has also been demonstrated. In *Homarus*, developed and functional effector organs such as gills coexist from stage IV with the neuroendocrine control of hydromineral metabolism. This could explain the establishment of the adult type of osmoregulation after metamorphosis. Different studies are underway to examine these hypotheses.

Relation between osmoregulation and salinity tolerance

Larvae of *H. americanus* and *P. japonicus* are weak regulators and their salinity tolerance is comparatively moderate or low. At metamorphosis, salinity tolerance is minimum while the pattern of osmoregulation changes. In post-larvae, the ability to osmoregulate increases either quickly (*Homarus*) or progressively (*Penaeus*) and so do their respective salinity tolerances. Thus, in both species, there is a strong correlation between increased ability to osmoregulate, in particular to hyper-regulate in low salinities, and improved salinity tolerance.

This implies a relationship between salinity-tolerant hyper-regulating stages on one hand and the probability that they will encounter low-salinity media on the other. This relation is not obvious in the case of *Homarus*, the different stages of which live in coastal waters where the salinity may fluctuate. Hyper-osmoconforming larvae could then rely on isosmotic intracellular regulation as they remain at the surface as planktonic stages, while, according to an hypothesis of Foskett (1977), the higher-density hyper-osmotic post-larval stages would be adapted to seek and settle on the bottom. The above mentioned relation is more evident in *P. japonicus*, in which sea-dwelling larvae hyper-osmoconform while, after their coast-bound migration, post-larvae living in coastal or estuarine environments and thus submitted to salinity fluctuations, strongly hyper-hypo-regulate and are widely tolerant of low salinities. As in the lobster, the stages during which hyper-regulation becomes fully established, *i.e.*, PL5/PL6, are also the stages of bottom settlement (Hudinaga, 1942), which enhances Foskett's hypothesis. Dall (1981) and Castille and Lawrence (1981) found in different species of penaeid shrimp that, compared to sea-living adults, coastal and estuarine juveniles displayed stronger hyper-regulation and better tolerance of low salinity. Such is also the case in *P. japonicus* (Charmantier *et al.*, unpub. data). Thus, throughout the development of some species of penaeid shrimp, osmoregulation and ecology are closely linked, although other factors such as bottom selection, food availability, and presence of predators may interfere with the choice of the biotope (Dall, 1981). In two other species in which the young stages encounter salinity extremes in their environment, *Macrobrachium petersi* (Read, 1984) and *Uca subcylindrica* (Rabalais and Cameron, 1985), a clear correlation has also been demonstrated between osmoregulatory ability and salinity tolerance.

In conclusion, the various studies conducted on young crustaceans reveal different patterns of ontogeny of osmoregulation. In one group of brachyuran species mentioned by Foskett (1977), osmoregulation varies little with developmental stage and no general trend can be found. In *M. petersi* (Read, 1984), which migrate between fresh and saline water for reproduction, the adult type of regulation is established as early as the first larval stage. In a third group of species, like *U. subcylindrica* (Rabalais and Cameron, 1985), *H. americanus*, and *P. japonicus* (this study), metamorphosis marks the appearance of the adult type of regulation. Further studies on other species should complement these ontogenetical groups. Since survival in low salinities appears correlated with the ability of *H. americanus* and *P. japonicus* to hyper-regulate in young stages, additional evidence of a relationship between the osmoregulatory ability, salinity tolerance and ecology of young stages of crustaceans should come from future studies.

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